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Chromium speciation in human blood samples based on acetyl cysteine by dispersive liquid–liquid biomicroextraction and in-vitro evaluation of acetyl cysteine/cysteine for decreasing of hexavalent chromium concentration

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ABSTRACT

A rapid and efficient method based on ionic liquid dispersive liquid–liquid biomicroextraction (IL-DLLBME) was used for speciation and preconcentration of Chromium (III, VI) in human blood samples before determination by electro-thermal atomic absorption spectrometer (ET-AAS). In this method, 1-hexyl-3-methylimidazolium hexafluorophosphate as a ionic liquid was dissolved in acetone as a dispersant solvent and then the binary solution was rapidly injected by a syringe into the blood samples containing Cr^{III}, which have already complexed by acetyl cysteine (NAC) at optimized pH. Under the optimal conditions, the linear range (LR), limit of detection (LOD) and preconcentration factor (PF) were obtained 0.03–4.4 $\mu g\,L^{-1}$, 0.005 $\mu g\,L^{-1}$ and 10 respectively (RSD <5%). In vitro study show us, the cysteine (Cys) as a prodrug of NAC can decrease the concentration of Cr(VI) in blood samples and human body. Validation of methodology was confirmed by standard reference material (SRM).

1. Introduction

Recently, heavy metals get distinguished from other toxic pollutants, due to their non-biodegradability can accumulate in living tissues, thus becoming concentrated throughout the food chain and can be readily absorbed by the human body. Even a very small amount of them can cause severe physiological or neurological damage to the human body. Cr is a major pollutant in the environment, usually as a result of some industrial pollution, including tanning factories, steelworks, industrial electroplating and wood preservation. Among the elements that currently need to be determined in environmental and pharmaceutical products, Cr is important in view of some health risks such as chromosomal aberration, mutations, carcinogenicity, transformation in cultured cells and a variety of DNA lesions [1].

Cr species exist mainly in two different oxidation states in the environment, Cr^{III} and Cr^{VI}, which have contrasting physiological effects. Cr^{III} compounds play an important role in the metabolism of glucose and certain lipids; in addition, it is considered an essential trace element for the maintenance of an effective protein metabolism in humans [2]. On the contrary, the Cr^{VI} toxic and carcinogenic effects present are due to their strong oxidation potential and their relatively small size, which enables them to penetrate through biological cell membranes, providing damage to macromolecules, proteins and DNA (DNA-Cr-DNA crosslinks). Cr^{VI} inhibits the enzymatic sulfur uptake of the cell and is also harmful to lungs, liver and kidneys [3].

Cr can enter the human body through breathing or drinking water, and its level in the air, water and biological samples is very low. Cr concentration in drinking water is generally less than 2 $\mu g\,L^{-1}$ [4]. The World Health Organization (WHO) states that the guideline values of 50 $\mu g\,L^{-1}$ Cr^{VI} are considered to be too high as compared to its genotoxicity, and the national health and nutrition examination survey claims normal Cr levels found in the human blood are 0.1–1.7 $\mu g\,L^{-1}$ and 0.24–1.8 $\mu g\,L^{-1}$ in urine [5]. Cr^{VI} compounds, once inside the bloodstream, are actively transported into red blood cells (RBC) via nonspecific anionic channels and then rapidly reduced to Cr^{III} which becomes bound to hemoglobin. Hexavalent chromium (Cr(VI)) is reduced intracellularly to Cr(V), Cr(IV) and Cr(III) by ascorbate (Asc), cysteine and glutathione (GSH). These

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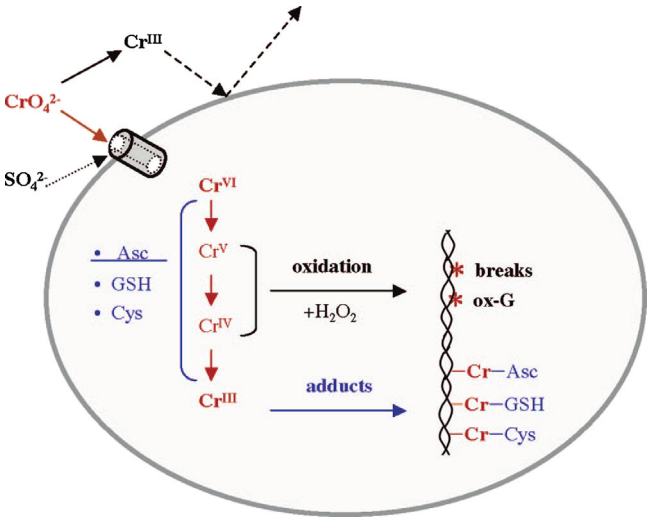

Fig. 1. Metabolism, and formation of DNA damage by Cr^{VI} at pH 7.4.

metabolites induce a spectrum of genomic DNA damage resulting in the inhibition of DNA replication (Fig. 1). Therefore, Cr levels in the red blood cells indicate exposure to Cr^{VI} and can be changed to Cr^{III} within these cells [6]. Chromate (CrO_4^{2-}) and hydrochromate ($HCrO_4^-$) are the main aqueous forms of Cr^{VI} at neutral pH and octahedral arrangement of H_2O groups in hexacoordinate complexes of Cr^{III}. At neutral pH, the $Cr(H_2O)_6^{3+}$ complex undergoes rapid hydrolysis, producing a mixture of mononuclear and polynuclear species containing hydroxo ligands [7]. In order to provide a timely warning of Cr exposure, it is highly desirable to develop suitable procedures for Cr speciation.

Sensitive techniques for determination of Cr species, including ion chromatography, inductively coupled plasma mass spectrometry [8], luminescence quenching [9], stripping voltammetry [10], flame atomic absorption spectrometry (F-AAS) [11], neutron activation analysis (NAA) [12], inductively coupled plasma optical emission spectrometry (ICP-OES) [13], ion chromatography inductively coupled plasma-mass spectrometry (IC-ICP-MS) [14] and electro-thermal atomic absorption spectrometry (ETAAS) [15,16] analysis techniques that were frequently coupled with a prior pre-concentration and/or separation steps. However, the high instrumental and operational costs and the high detection limits are common disadvantages of many of these methods. Sample preparation procedures such as liquid–liquid extraction (LLE) [17], homogeneous liquid–liquid extraction [18], solid phase extraction (SPE) [19], liquid-phase micro-extraction (LPME) [20] and Cloud point extraction (CPE) [21] are developed to simplify analytical approaches as it reduces costs.

Dispersive liquid–liquid micro-extraction (DLLME) is a miniaturization of the traditional LLE technique, where the extractant phase is a drop of a few microlitres of a water-immiscible solvent that can be directly immersed in the sample and dispersed by organic solvent. Although organic solvents (i.e., octanol, cyclohexane and toluene) are useful as extractant phase, but these solvents are toxic, recently the use of ionic liquids (ILs) has been proposed in most LPME modes. They have various advantages over traditional organic solvents, such as low vapour pressure, high stability, large viscosity, adjustable miscibility and polarity, good extractability for different organic and inorganic compounds [22,23].

Acetyl cysteine, acetamido sulfanylpropanoic acid, (2R)-2-acetamido-3-sulfanylpropanoic acid or NAC is a pharmaceutical drug and nutritional supplement used as a mucolytic agent, acetaminophen overdose and autism [24]. It is a derivative of cysteine with an acetyl group attached to the amino group of cysteine. NAC is essentially a prodrug that is converted to cysteine (in the intestine by the enzyme aminoacylase) and absorbed in the intestine into the blood stream. NAC is on the WHO's list of essential medicines, a list of the most important medication needed in a basic health system [25].

In this work, in vitro speciation and preconcentration of chromium (III, VI) in human blood samples based on NAC were obtained by IL-DLLBME. In additon, the effect of Cys as an essentially product of NAC on decreasing of Cr(VI) concentration in human blood samples were in-vitro investigated. Experimental parameters affecting the extraction process were optimized and the performance of the proposed method was evaluated.

2. Experimental

2.1. Apparatus

Determination of Cr was performed with spectra GBC electrothermal atomic absorption spectrometer (Plus 932, Australia) using a graphite furnace module (GF3000, GBC). The operating parameters for metal of interest were set as recommended by the manufacturer. A hollow cathode lamp (GBC) operated at a current of 6 mA and a wavelength of 357.9 nm with a spectral band width of 0.2 nm was used. All experiments were performed by using a sample volume of 20 μL by auto-sampler injector of ET-AAS. As a cross-validation of methodology, inductivity coupled plasma mass spectrometry (ICPMS) was used for determination of ultra-trace chromium in standard and human samples (Perkin Elmer, QP, Elan6000 DRC, RIPI, Iran). The pH values of the solutions were measured by a digital pH meter (Metrohm 744). The instrumental conditions for ETAAS have explained in part of analytical figures of merit.

2.2. Reagents

All reagents were of ultra-trace analytical grade from Merck (Germany) and Aldrich (Germany). Cr(III) stock solution was prepared from an appropriate amount of the nitrate salt of this analyte as 1000 mg L^{-1} solution in 1% HNO_3 (Merck). Cr(VI) stock solution was prepared from an appropriate amount of the 1 g of Potassium salt ($K_2Cr_2O_7$) of this analyte as 1000 mg L^{-1} solution in 1% HCl. Standard solutions were prepared daily by dilution of the stock solution in nitric acid. Ultra-pure lithium heparin (H0878, CAS N: 9045-22-1,100KU) and sodium citrate (S4641, CAS N: 6132-04-3, 25 g) were purchased from Sigma–Aldrich in Iran. The pH adjustments of samples were made using appropriate buffer solutions including sodium acetate (CH_3COONa/CH_3COOH, 1–2 mol L^{-1}) for pH 3–7, and ammonium chloride (NH_3/NH_4Cl, 0.2 mol L^{-1}) for pH 8–11. Polyoxyethylene octyl phenyl ether (TX-100) and (2R)-2-acetamido-3-sulfanylpropanoic acid (NAC, $C_5H_9NO_3S$) were also purchased from Sigma–Aldrich. Ultrapure water (18 MΩ cm) was obtained from Millipore Continental Water System (Bedford, USA), and 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF_6]) was prepared from Sigma–Aldrich (50 g, Germany).

2.3. Human biological sampling

Heparin and citrate are commonly used as anticoagulants in human blood samples. The blood collection tube with citrate or heparin was aliquoted into Eppendorf (2 mL) tubes and kept at −20 °C for one week. Citrate chelates calcium (Ca^{2+}) a critical factor of coagulation and heparin binds to several enzymes responsible for the coagulation cascade. For sampling, all glass tubes were washed with a 0.5 mol L^{-1} HNO_3 solution for at least 24 h and thoroughly rinsed 6 times with ultrapure water before use. As Cr concentrations in whole blood and serum are very low, even minor